Activation of neutrophils by plasma derived FVIII concentrates

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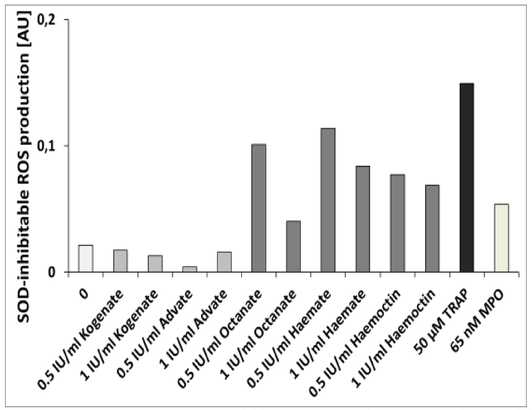
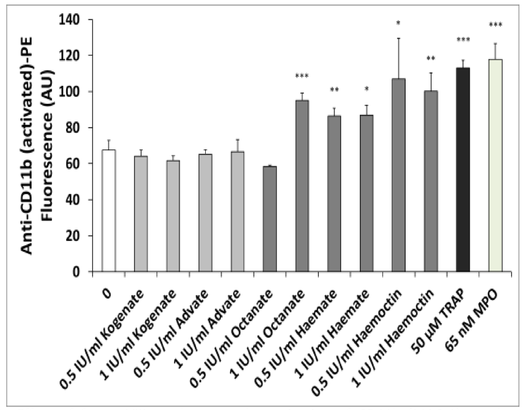
Introduction: In previous projects we showed that pdFVIII activated platelets and induced cellular stress in various cell types (platelets, monocytes, endothelial cells, synovial cells). In this project, we studied whether pdFVIII may boost inflammation by the activation of neutrophils.

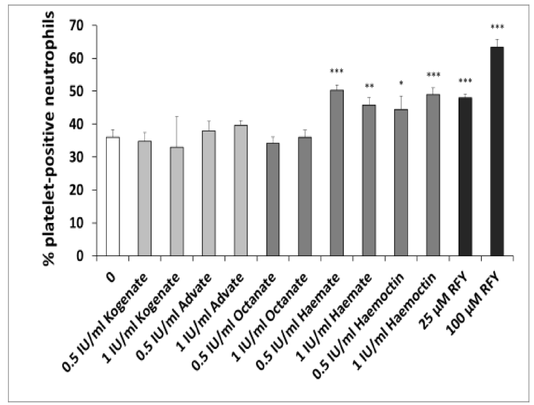
Methods: Three pdFVIII products (Octanate®, Octapharma AG, HaemateP®, CSL Behring, and HaemoctinSDH®, Biotest) and two rFVIII products (Kogenate®Bayer and Advate®, Baxter) were studied. Neutrophils were gently isolated from fresh human blood by counter-flow *elutriation*. Whether FVIII concentrates induced generation of the reactive oxygen species (ROS) superoxide anion ($O_2^{\cdot-}$) from nonactivated neutrophils was determined as the linear rate of superoxide dismutase-inhibitable reduction of cytochrome C. Activation of CD11b (MAC-1) was analyzed by flow cytometry using an activation specific monoclonal antibody labeled with PE. Associate formation in whole human blood between platelets and neutrophils was measured by flow cytometry. Effect of factor VIII products on DNA release from isolated human neutrophils was measured by a fluorogenic assay using Syto13, a fluorescent dye binding nucleic acids, and a sandwich ELISA against histone-DNA complexes (Roche) detecting neutrophil extracellular traps (NETs) from FVIII treated human neutrophils.



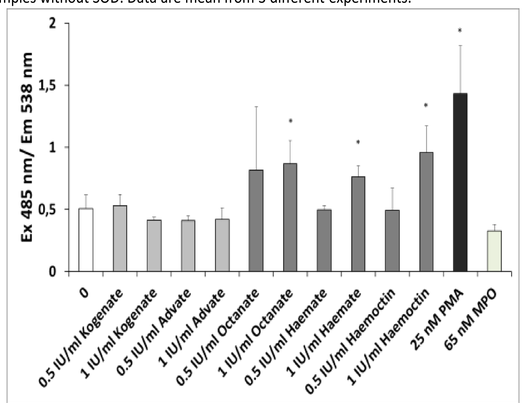
Effect of factor VIII products on the SOD (superoxide dismutase) -inhibitable ROS production of isolated human neutrophils. Isolated human neutrophils were incubated for 45 min at 37 °C with 0.5 or 1 IU/ml of the indicated factor VIII product, 50 µM TRAP, 65 nM MPO or buffer alone, respectively. Afterwards, 100 µM cytochrome c was added to all samples and 12.5 µg/mL SOD was added to half of the samples. After 10 min of incubation, absorption at 550 nm was measured. SOD-inhibitable ROS production was calculated by substracting the absorption of samples with SOD from the absorption of samples without SOD. Data are mean from 3 different experiments.



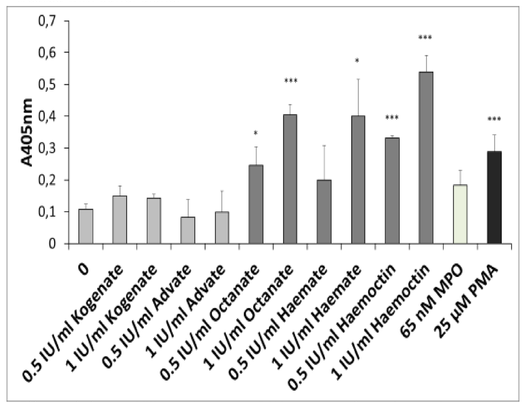
Effect of factor VIII products on the activation of CD11b (MAC-1) on the surface of isolated human neutrophils. Isolated human neutrophils were incubated for 45 min at 37 °C with 0.5 or 1 IU/ml of the indicated factor VIII product, 50 µM TRAP, 65 nM MPO or buffer alone, respectively. Afterwards, anti-activated CD11b-PE and neutrophil-specific anti-CD66b-FITC were added and cells were analyzed by flow cytometry. Data are mean ± SD from 3 different experiments. *** p<0.005, ** p<0.01, * p<0.05 compared to cells treated with buffer alone.



Effect of factor VIII products on the formation of heterotypic associates between platelets and neutrophils in melagatran-anticoagulated whole blood. Melagatran (3 µM) anticoagulated whole blood was incubated for 45 min at 37 °C with 0.5 or 1 IU/ml of the indicated factor VIII product, 25 or 100 µM RFY or buffer alone, respectively. Afterwards, platelet-specific anti-CD42a-PE and neutrophil-specific anti-CD66b-FITC were added. After 20 min of incubation, cells were fixed, erythrocytes were lysed and samples analyzed by flow cytometry. Data are mean ± SD from 3 different experiments. *** p<0.005, ** p<0.01 compared to cells treated with buffer alone.

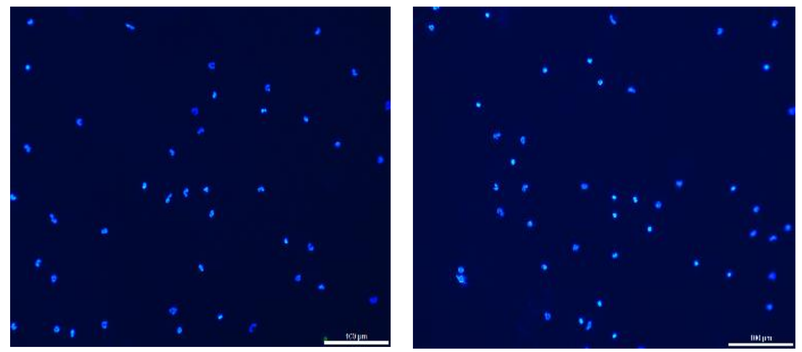


Effect of factor VIII products on DNA release from isolated human neutrophils measured by a fluorogenic assay. Isolated human neutrophils were incubated for 3 h at 37 °C with 0.5 or 1 IU/ml of the indicated factor VIII product, 25 nM PMA, 65 nM MPO or buffer alone, respectively. Afterwards, cells were harvested; supernatant was transferred to a black 96 well plate and 1 µM Syto 13 was added. After 5 in of incubation, fluorescence was measured using the Fluososkan Ascent fluorimeter (Excitation 485 nm, Emission 538 nm). Data are mean ± SD from 3 different experiments. *** p<0.005, ** p<0.01, * p<0.05 compared to cells treated with buffer alone (control).



Effect of factor VIII products on DNA release from isolated human neutrophils measured by ELISA. Isolated human neutrophils were incubated for 3 h at 37 °C with 0.5 or 1 IU/ml of the indicated factor VIII product, 25 nM PMA, 65 nM MPO or buffer alone, respectively. Afterwards, cells were harvested and supernatant was analyzed regarding extracellular nucleosomes using the Cell Death Detection ELISA from Roche. Data are mean ± SD from 3 different experiments. *** p<0.005, ** p<0.01, * p<0.05 compared to cells treated with buffer alone (control).

References: Brodde et al. 2014, Transfus Med Hemother, 41:140-144
Brodde et al. 2010, Transfus Med Hemother, 37:175-184

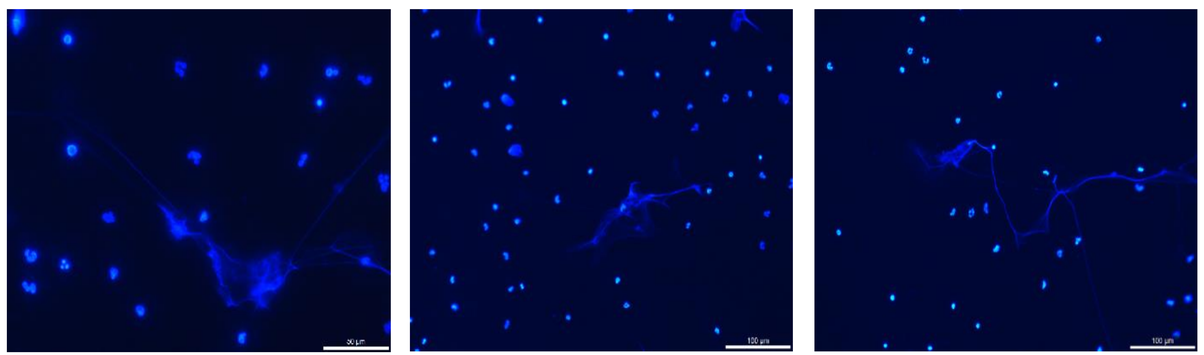


rFVIII: Kogenate rFVIII: Advate

No NET formation after incubation of fresh isolated human neutrophils for 60 minutes with 1 IU/ml recombinant factor VIII.

Cells were fixed and coloured with Hoechst 33324, a fluorescent stain for DNA (blue).

NET formation after incubation of fresh isolated human neutrophils for 60 minutes with 1 IU/ml pdfactor VIII.



pdFVIII: Octanate pdFVIII: Haemate pdFVIII: Haemoctin

Results: Significant activation of CD11b (MAC-1) was only observed on neutrophils treated with (0.1-1 IU/ml) pdFVIII, but not on neutrophils treated with rFVIII. pdFVIII induced neutrophil ROS production as well as the release of extracellular DNA (neutrophil extracellular traps, NETs), which was not observed with rFVIII. In addition to experiments with isolated neutrophils, we studied the effect of FVIII on neutrophils in melagatran anticoagulated whole blood. Two of three tested pdFVIII clearly and significantly induced the formation of associates between neutrophils and platelets. In contrast, rFVIII had no effect. The activation effects of pdFVIII on neutrophils were more pronounced in the presence of platelets.

Conclusions: Based on these in vitro results, pdFVIII products, in contrast to rFVIII products, seem to be proinflammatory by activating neutrophils.

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The poster was presented on the WFH 2016 in Orlando, USA